UTILIZATION OF DISSOLVED EXOGENOUS NUTRIENTS BY THE STARFISHES, ASTERIAS FORBESI AND HENRICIA SANGUINOLENTA

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In the past few years it has become increasingly evident that nutrition in many types of invertebrate animals involves not only the ingestion of solid foods or particulate matter, but also the utilization of dissolved organic materials commonly found in the environment. While speculation on the significance of this latter source of nutrients dates back at least to the work of Pütter (1909), it remained for Stephens and Schinske (1961) to provide the first clear evidence that dissolved materials can be taken up by a wide variety of invertebrates. These workers demonstrated that representatives of 10 phyla (including Echinodermata) could remove glycine from dilute solutions in sea water. Stephens has continued his investigations and further described the uptake of dissolved amino acids and sugars by several forms, notably the coral *Fungia* (Stephens, 1962), various annelids (Stephens, 1963, 1964), and brittle stars (Stephens and Virkar, 1965, 1966).

In the course of my own studies (Ferguson, 1963a, 1963b), I have observed, by the use of autoradiographic methods, that dissolved C^{14}-labeled nutrients (glucose and amino acids) appear to be readily taken up into at least the epidermal tissues of *Asterias forbesi.* I have suggested that this may represent the most important source of nutrients to some of the more isolated superficial tissues of starfishes, and that in species such as *A. forbesi* the epidermal absorptive process may be facilitated "by enrichment of the medium with stray products released from the externally digested food and by scavenging activities of pedicellariae" (Ferguson, 1963a, p. 79).

Most recently, Pequignat (1966) has reported detailed investigations on a number of echinoderms, including *Asterias rubens,* demonstrating digestion of various types of nutritional products on the skin by glandular secretions and migrating coelomocytes. While his observations are basically subjective in nature, he concludes that at least some of the materials which are digested externally are absorbed directly into the epidermis.

At this time, then, it appears that dissolved organic materials are utilized by starfish (and many other invertebrates), and that at least some of the nutrients are taken up directly by the body surface, thus by-passing the digestive tract. Furthermore, it is probable that in various species of echinoderms mechanisms, such as the pedicellariae, have evolved which serve to enhance the availability of dissolved nutrients to the integuments. There are, however, at least several important questions which are as yet unanswered. First, are dissolved nutrients taken up by the digestive tract as well as the epidermis? Second, do epidermally absorbed nutrients

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become distributed throughout the body, or can they benefit only the superficial tissues into which they are initially taken up? And third, are there marked differences in the handling of exogenous nutrients by various species of starfishes? The present investigation has been directed toward these three points.

Materials and Methods

The starfish used in these experiments were freshly collected specimens of *Asterias forbesi* and *Henricia sanguinolenta* obtained from the Supply Department of the Marine Biological Laboratory. A few specimens of *Asterias vulgaris* were also studied, but as these did not appear to react differently from *A. forbesi* further work on this species was not continued. All the animals used were about 2 inches in diameter. They were placed individually in beakers containing a medium consisting of 50 ml. of filtered sea water and dissolved, C^{14}-labeled nutrients. The specimens were left in this medium for a period of 8 hours (except those sacrificed at 1 hour), and then rinsed twice and placed in a holding tank of running sea water. While retained in the holding tank they were provided with a number of small clams to serve as food. The distribution of radioactivity in the tissues of groups of animals was analyzed following periods of 1, 8, and 72 hours, and 20 days, measured from the time the animals were first placed in the medium.

Two types of medium were used. One consisted of 0.5 microcurie (0.0033 mg.) of a mixture of 15 uniformly C^{14}-labeled, purified amino acids per 50 ml. of filtered sea water. The manufacturer of the amino acid mixture (New England Nuclear Corp. of Boston, Mass.) claims that it contains the "same relative proportions as found in a typical algal protein hydrolysate." The other medium consisted of 0.5 microcurie (1.85 mg.) of uniformly labeled C^{14}-glucose in each 50-ml. portion.

In order to measure the distribution of the labeled nutrients in the animals, each specimen was dissected as follows: the rays were cut off as near to the disk as possible. Incisions were then made up the lateral edges of each ray so that the oral and aboral portions could be separated. Next, the digestive glands were pulled free from the aboral portion. The disk was then picked up and each of its supporting columns severed so that it could be opened and the stomach (both cardiac and pyloric divisions) cut free.

As a result of this procedure five groups of tissue were obtained. These will be referred to as the "disk," "oral body wall," "aboral body wall," "stomach," and "digestive glands." The gonads were always included with the disk group, as their state of development was not consistent enough to warrant a separate set of analyses. Furthermore, preliminary studies had demonstrated negligible uptake of the nutrients by these structures.

The groups of tissues were then processed in two different ways for analysis of their radioactivity. The first method was designed to measure the total amount of material actually absorbed and retained. In it, the tissues were digested at 100° C. in test tubes with 1 ml. of 1 *M* NaOH in sea water. Digestion was enhanced by adding 1 or 2 drops of 30% H_2O_2. When all of the soft tissues were uniformly dispersed, the contents of the tubes were decanted into tared, 1-inch, stainless steel sample pans and dried in an oven. The radioactivity of each sample was measured in a Nuclear-Chicago, low-background, G-M counter fitted with a "Micromil" window. Corrections were made on the basis of infinite thickness and the counts

compared to those of similarly prepared tissues to which known quantities of labeled nutrients had been added. The corrected measurements of 27 such standard samples had a mean deviation of 11.6%. The alkaline digestion was used in preference to solubilizing in acid as it prevented the loss of carbonaceous endoskeletal material.

The second method was intended to determine the amounts of absorbed nutrient materials which were retained by the tissues in a relatively unbound state. In this procedure, each group of tissue was extracted 48 hours in 10 ml. of ethanol solution. Based on the results of test runs, an 80% concentration of alcohol was found most satisfactory for the amino acid samples and a 40% solution best for the samples containing glucose. In both cases, duplicate 0.25-ml. aliquots of the extracts were plated onto $1\frac{1}{4}$-inch stainless steel sample pans, dried, and counted. Again, the counts were compared to those of samples to which known quantities of tracers had been added. The counts of 30 standard samples exhibited a mean deviation of 6.7%.

Two to 5 specimens of each starfish species were treated by both methods for each of the 8 different combinations of time interval and type of medium employed.

Results

Quantity of nutrients taken up

Almost all the animals used in the study absorbed significant amounts of the labeled nutrients made available to them. In the experiments involving the amino

Table I

Distribution of exogenous amino acids taken up by starfish tissues.
(Expressed as % of initial quantity to which animals were exposed)

Time	Species	Disk		Oral body wall		Aboral body wall		Stomach		Digestive gland		Total	
		Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%
1 hour	*H. sang.*	1.85	8.1	1.54	19.0	0.72	10.8	0.23	0.1	0.47	0.2	4.81	38.2
	H. sang.	0.69	4.3	1.40	27.2	0.63	8.5	0.11	0.1	0.32	0.1	3.15	40.2
	A. forb.	0.70	11.2	1.26	25.0	0.67	26.8	0.06	0.1	0.40	0.1	3.09	63.2
	A. forb.	1.35	13.7	2.89	34.0	1.79	10.1	0.08	0.0	0.48	0.1	6.59	57.9
8 hours	*H. sang.*	0.51	10.3	1.37	27.1	0.76	11.7	0.07	0.1	0.17	0.1	2.88	49.3
	H. sang.	1.16	10.3	2.91	21.0	1.17	17.9	0.20	0.2	1.41	0.4	6.85	49.8
	A. forb.	0.79	6.7	1.12	21.4	0.56	9.6	0.13	0.1	0.36	0.2	2.96	38.0
	A. forb.	1.01	9.1	1.57	18.7	0.74	10.7	0.22	0.1	0.49	0.1	4.03	38.7
72 hours	*H. sang.*	1.01	11.5	1.41	32.3	0.59	15.9	0.46	0.2	0.70	0.5	4.17	60.4
	H. sang.	0.95	14.6	1.44	37.5	1.53	11.7	0.27	1.6	0.35	1.9	4.54	67.3
	A. forb.	1.18	9.0	3.15	23.3	2.67	17.4	0.11	0.1	0.80	0.7	7.91	50.5
	A. forb.	1.52	13.4	4.15	22.0	1.80	20.2	0.35	0.1	1.62	0.9	8.44	56.6
20 days	*H. sang.*	0.46	9.3	0.49	25.2	0.24	14.2	0.11	0.3	0.11	0.5	1.41	49.5
	H. sang.	0.86	11.0	0.69	25.2	0.36	27.4	0.17	0.2	0.22	0.4	2.30	64.2
	H. sang.	0.69	12.7	0.85	27.3	0.43	20.6	0.11	0.3	—	0.5	2.08+	61.4
	A. forb.	0.72	16.0	1.48	27.7	0.64	21.1	0.09	0.1	0.44	0.4	3.37	65.3
	A. forb.	0.77	12.9	0.48	35.1	0.77	14.5	0.08	0.1	0.40	0.4	2.50	63.0
	A. forb.	0.69	17.2	1.41	25.8	0.81	18.0	0.05	0.1	0.24	0.4	3.20	61.5

TABLE II

Distribution of exogenous glucose taken up by starfish tissues.
(Expressed as % of initial quantity to which animals were exposed)

Time	Species	Disk		Oral body wall		Aboral body wall		Stomach		Digestive gland		Total	
		Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%
1 hour	*H. sang.*	0.86	0.2	1.22	0.4	0.51	0.2	0.14	0.0	0.34	0.0	3.07	0.8
	H. sang.	1.10	0.3	1.49	0.7	0.58	0.4	0.24	0.0	0.33	0.1	3.74	1.5
	A. forb.	0.55	1.2	1.16	3.2	0.69	0.8	0.13	0.6	0.57	0.1	3.10	5.9
	A. forb.	1.14	2.1	2.04	5.7	1.19	2.1	0.21	0.0	1.36	0.1	5.94	10.0
8 hours	*H. sang.**	1.04	7.8	1.42	3.5	0.81	6.6	0.20	6.5	0.38	14.5	3.85	38.9
	*H. sang.**	0.96	7.6	1.47	2.5	0.71	3.1	0.23	13.9	0.46	38.6	3.83	65.7
	A. forb.	1.11	8.5	2.60	27.3	2.09	13.7	0.09	0.7	0.58	0.2	6.47	50.4
	A. forb.	1.34	7.1	3.16	27.9	2.21	8.8	0.18	0.1	1.07	0.2	7.96	44.1
72 hours	*H. sang.**	0.94	2.6	1.51	5.8	0.81	3.8	0.27	1.3	0.59	4.7	4.12	18.2
	H. sang.	1.15	1.2	1.92	7.3	0.97	2.7	0.16	0.1	0.55	0.1	4.75	11.4
	H. sang.	0.89	0.9	0.99	1.5	0.47	0.8	0.20	0.1	0.24	0.2	2.79	3.5
	*H. sang.**	0.77	0.9	1.30	1.5	0.84	1.7	0.10	0.6	0.31	1.2	3.32	5.9
	A. forb.	0.92	4.7	3.44	13.5	2.56	23.0	0.16	0.1	0.95	0.4	8.03	41.7
	A. forb.	0.88	9.1	1.97	34.4	1.21	6.9	0.10	0.1	0.67	0.2	4.83	50.7
20 days	*H. sang.**	0.73	1.6	0.80	1.3	0.41	0.6	0.08	1.4	0.12	1.1	2.14	6.0
	*H. sang.**	0.69	2.3	0.71	1.3	0.82	1.2	0.11	3.0	0.17	4.0	2.50	11.8
	H. sang.	0.63	0.6	0.61	1.1	0.32	0.5	0.11	0.1	0.13	0.2	1.80	2.5
	*H. sang.**	0.22	0.8	0.34	0.8	0.22	0.6	0.03	1.8	0.05	2.2	0.86	6.2
	H. sang.	0.32	0.3	0.38	0.6	0.21	0.5	0.05	0.1	0.10	0.1	1.06	1.6
	A. forb.	0.46	3.4	1.23	10.5	0.89	2.9	0.08	0.1	0.30	0.1	2.96	17.0
	A. forb.	0.95	6.6	1.84	25.8	0.98	6.8	0.16	0.3	0.74	0.3	4.67	39.8
	A. forb.	0.68	4.0	1.85	12.5	1.38	5.2	0.04	0.1	0.46	0.1	4.41	21.9

acid mixture (Table I), usually about 40 to 65% of the radioactive elements initially present was removed. Interestingly, most of this uptake appeared to take place during the first hour of incubation. In fact, with *Asterias,* the mean total values for absorption were less after 8 hours than they were after 1 hour (Fig. 1). Considering the variation between the different specimens, however, this apparent decrease probably would not have been observed if a larger number of animals had been tested.

Nevertheless, a very large proportion of the total uptake of the amino acids did take place with both species early in the incubation period. While the causes of this effect are uncertain, the property could have been due to at least two factors. Firstly, certain of the types of the amino acids included in the mixture presumably are more easily absorbed than others, and thus, these types would become rapidly depleted from the medium. The less-easily absorbed amino acids remaining after the first hour would be taken up more slowly over a longer period of time. Secondly, the organism could release substances which would accumulate in the sea water and, after an interval, some of these might reach concentrations sufficient to inhibit the

absorption of the amino acids which had not yet been taken up. Such an inhibition would be relatively easy to achieve considering the small quantities of labeled amino acid used. Both of these phenomena have been observed in previous experiments

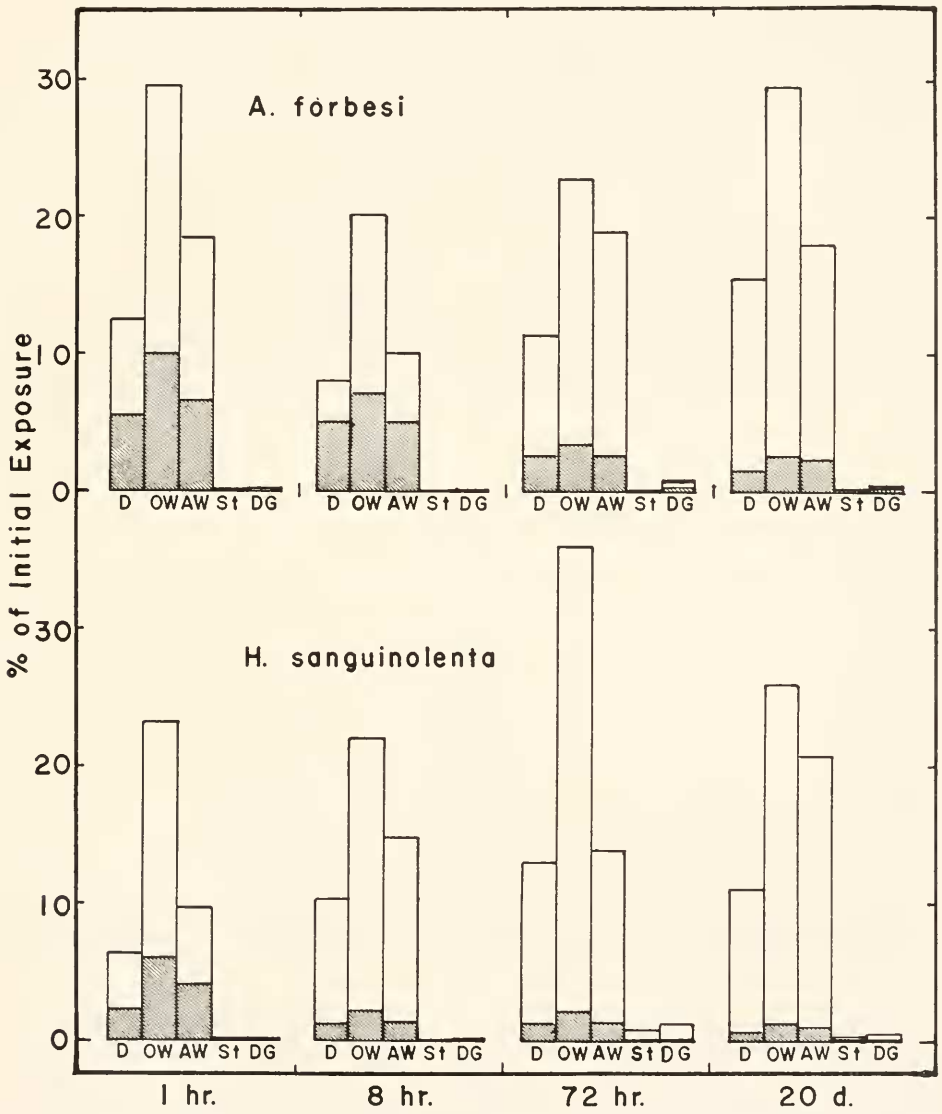

FIGURE 1. Quantities of absorbed amino acids found in five different body regions of specimens of two species of starfishes. Values (% of initial exposure) refer to the percentages of the total initial C^{14}-labeled amino acid present in the medium which were recovered from the different groups of tissues (mean 2–3 specimens). The entire bars represent the total uptake (digest method) while the cross-hatched areas represent material remaining unbound (alcohol extract method). D, disk; OW, oral body wall; AW, aboral body wall; St, stomach; DG, digestive glands. Over the 20-day period there is little redistribution of the absorbed amino acids. For further explanation, see text.

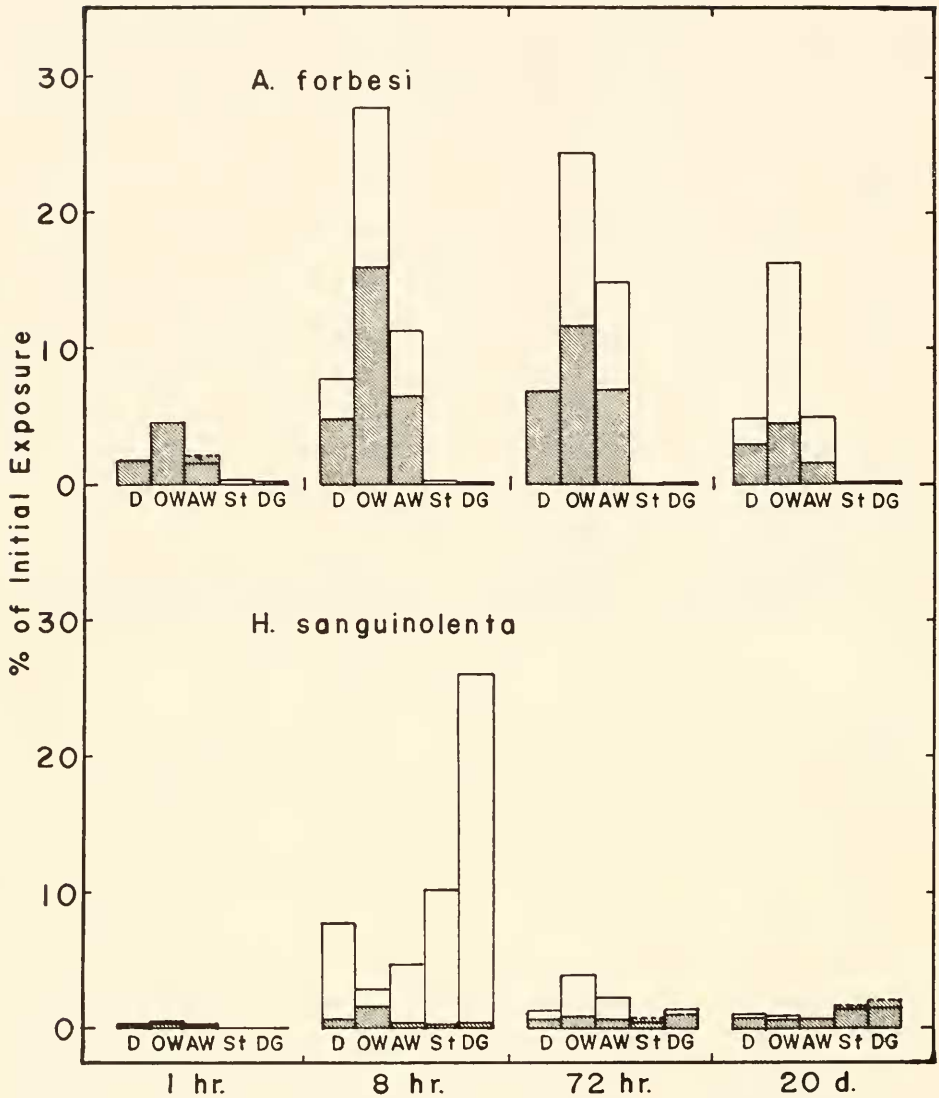

FIGURE 2. Quantities of absorbed glucose found in five different body regions of specimens of two species of starfishes. Values (% of initial exposure) refer to the percentages of the total initial C^{14}-labeled glucose present in the medium which were recovered from the different groups of tissues (mean 2–5 specimens). Symbols are the same as in Figure 1. Some specimens of *Henricia* apparently have taken up the glucose directly into their digestive organs. For further explanation, see text.

dealing with the uptake of amino acids by isolated starfish organs (Ferguson, 1964, and unpublished data).

In contrast to the time course of amino acid uptake which was observed, glucose apparently was absorbed continually over the 8-hour incubation period. This fea-

ture can be seen in Table II where the values for total per cent uptake by the 8-hour specimens are many times those of the 1-hour specimens. The glucose solution, unlike the amino acid mixture, was homogeneous. Also, the molar concentration of the glucose was considerably higher than that of the amino acids (because of its lower specific activity). Thus, while the percentages of glucose taken up appear to be somewhat lower than those of the amino acids, the actual quantities were probably much greater. Likewise, at the end of the incubation period the concentration of glucose still remaining in the medium was greater than even the initial concentration of amino acid used.

Distribution of the absorbed nutrients

With a few specific exceptions, practically all of the labeled nutrients which were taken up from the two types of medium were absorbed by the body wall components of the starfishes (Figs. 1 and 2). Very little (less than 1%) normally found its way into the internal organs. Even after 20 days there generally was no increase in the radioactivity of these structures which could be considered significant. The greatest quantities of the nutrients were most often found in the oral portions of the body wall. These substances were probably absorbed by the extensive surface of the tube feet and other areas of the epidermis of this region.

The mean values for the distribution of the glucose absorbed by *Henricia* (Fig. 2) present a pattern markedly different from that observed in the other cases. Indeed, in looking at the 8-hour specimens, the distribution is seen to be almost completely reversed; the least activity is found in the oral body wall and the greatest in the digestive glands. A study of the actual data which were recorded (Table II) helps to clarify what has happened. A number of the specimens of *Henricia* (marked *) show large values for the percentages of material taken up into their internal organs and low ones for the uptake into external parts. Other individuals of the species exhibit the opposite distribution and in this sense more closely resemble the specimens of *Asterias*. It appears, then, that the marked specimens responded to some stimulus, probably the relatively high glucose concentrations employed, by initiating a kind of feeding reaction in which the dissolved nutrient was removed from the medium by the internal digestive organs. The same phenomenon can also be noted in the data for some of the specimens which were extracted with alcohol (Table IV), but since the values recorded from these analyses are quite a bit lower, the differences do not stand out as pronouncedly.

Loss of nutrients taken up

After the completion of the 8-hour incubation period, there was little change in the total amino acid radioactivity observed in the various specimens (Fig. 1, Table I). Apparently, the tissues had a strong affinity for the amino acids once they had taken them up, and over the 20-day period did not release them back into the sea water or lose them through metabolism and respiration to any significant degree.

There was, however, a very marked loss of radioactive glucose from animals over the same period (Fig. 2, Table II). This reduction was most obvious in *Henricia*, but clearly also took place in the specimens of *Asterias*. While no evidence was obtained relative to the fate of this lost material, it most probably dis-

appeared through the breakdown of the sugar by the cells and its release as respiratory CO_2.

Utilization of the absorbed nutrients

The analyses of the alcoholic extracts of the experimental animals provide data (Tables III and IV) through which additional insight may be gained into the ways in which the absorbed nutrients are utilized. This method measures only the labeled material which remains in a relatively "unbound" state after it is taken up.

TABLE III

Distribution of exogenous amino acids taken up by starfish tissues and retained in an unbound (alcohol-soluble) state. (Expressed as % of initial quantity to which animals were exposed)

Time	Species	Disk		Oral body wall		Aboral body wall		Stomach		Digestive gland		Total	
		Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%
1 hour	*H. sang.*	1.08	2.5	1.48	6.5	0.54	6.3	0.17	9.2	0.40	0.4	3.67	15.9
	H. sang.	0.82	1.9	1.18	5.6	0.54	1.8	0.09	0.1	0.16	0.1	2.79	9.5
	A. forb.	0.51	6.5	1.06	10.9	0.52	6.0	0.14	0.1	0.39	0.1	2.62	23.6
	A. forb.	1.03	4.6	1.94	8.8	1.74	7.0	0.04	0.1	0.68	0.0	5.43	20.5
8 hours	*H. sang.*	1.06	1.6	1.20	4.7	0.65	2.8	0.11	0.1	0.41	0.2	3.43	9.4
	H. sang.	1.24	1.6	2.53	3.4	1.23	2.6	0.71	0.1	0.12	0.1	5.83	7.8
	A. forb.	1.88	4.7	3.90	7.4	3.58	4.4	0.10	0.1	0.92	0.4	10.38	17.0
	A. forb.	3.21	5.4	5.10	6.6	3.29	5.6	0.29	0.2	0.99	0.0	12.88	17.8
72 hours	*H. sang.*	0.81	1.2	1.20	2.6	0.47	1.0	0.47	1.0	0.34	0.1	3.29	5.9
	H. sang.	1.36	1.3	1.49	1.7	0.70	1.9	0.16	0.2	0.27	0.1	3.98	5.2
	A. forb.	0.73	2.7	1.42	4.0	0.69	3.0	0.12	0.1	0.36	0.2	3.32	10.0
	A. forb.	2.51	2.4	3.30	2.9	1.64	2.3	0.33	0.0	1.04	0.1	8.82	7.7
20 days	*H. sang.*	0.43	0.7	0.78	1.5	0.30	0.9	—	0.2	0.01	0.2	1.52+	3.5
	H. sang.	0.36	0.6	0.42	1.2	0.18	0.9	—	0.1	0.01	0.1	0.97+	2.9
	A. forb.	1.10	1.5	1.67	2.1	1.23	2.5	0.16	0.2	1.07	0.3	5.23	6.6
	A. forb.	1.29	1.4	2.53	2.9	1.59	1.9	0.15	0.2	0.80	0.2	6.36	6.6

By comparison of these data with the results of the digestive method, an estimate can be obtained of the relative proportion of bound and unbound material retained by the cells at each period. These differences can be appreciated most easily with the aid of the two figures, by comparing the dark areas of each bar with the total length of the bar.

By such means it can be seen that, except for the initial periods, only a fraction of the material taken up normally was recoverable in the extracts. In the case of the amino acids (Fig. 1), the size of the soluble fraction decreased progressively in *Asterias* over the 20-day interval, from a peak at the end of the incubation period of 45% of the total amount absorbed to a low of 10% after 20 days. In *Henricia,* the range was from 32% at 1 hour to 5% at 20 days. It is interesting that in both

species over one-half of the absorbed amino acid was unextractable with alcohol after only a single hour of incubation. Apparently, some of the absorbed amino acid was bound up quite rapidly while the rest remained in a soluble pool in the cells and was incorporated into proteins or metabolized much more slowly. There could possibly be some exchange between the soluble pool and the bound state. If such an exchange does occur, it presumably would also prolong the apparent time required for the extractable fraction to diminish.

TABLE IV

Distribution of exogenous glucose taken up by starfish tissues and retained in an unbound (alcohol-soluble) state. (Expressed as % of initial quantity to which animals were exposed)

Time	Species	Disk		Oral body wall		Aboral body wall		Stomach		Digestive gland		Total	
		Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%	Wet wt. gm.	%
1 hour	*H. sang.*	0.70	0.1	1.49	0.4	0.59	0.2	—	0.0	0.27	0.0	3.05+	0.7
	H. sang.	1.50	0.1	2.02	0.3	0.87	0.2	0.16	0.0	0.41	0.0	4.96	0.6
	A. forb.	0.76	1.9	1.70	4.9	1.15	2.1	0.03	0.0	0.67	0.0	4.31	8.9
	A. forb.	1.61	1.5	3.98	4.1	2.78	2.1	0.17	0.1	1.27	0.0	9.81	7.8
8 hours	*H. sang.*	—	0.8	—	1.5	—	0.6	—	0.5	—	0.8	—	4.2
	H. sang.	—	0.7	—	1.8	—	1.3	—	0.1	—	0.1	—	4.0
	H. sang.	1.02	1.8	1.19	1.6	0.55	1.1	0.10	0.6	0.43	2.0	3.29	7.1
	H. sang.	1.92	0.5	1.84	1.0	1.11	0.8	0.22	0.2	0.73	0.2	5.82	2.7
	A. forb.	—	4.1	—	16.8	—	4.6	—	0.2	—	0.1	—	25.8
	A. forb.	—	5.7	—	15.1	—	8.5	—	0.0	—	0.1	—	29.4
72 hours	*H. sang.*	0.99	0.3	1.29	0.7	0.60	0.5	0.12	0.1	0.36	0.1	3.36	1.7
	H. sang.	1.66	1.3	2.02	1.3	0.79	0.9	0.29	2.6	0.50	3.2	5.26	9.3
	H. sang.	1.25	0.7	1.26	0.5	0.81	0.7	0.14	0.9	0.25	0.7	3.71	3.5
	H. sang.	1.20	0.5	1.59	1.1	0.91	0.9	0.21	0.1	0.36	0.3	4.27	2.9
	A. forb.	0.99	3.1	0.96	7.9	2.48	7.0	0.07	0.1	0.42	0.1	4.92	18.2
	A. forb.	1.86	8.4	3.34	15.8	1.79	7.0	0.16	0.1	0.93	0.2	8.08	31.5
20 days	*H. sang.*	0.91	0.6	1.24	0.4	0.54	0.3	0.23	0.7	0.36	0.6	3.28	2.6
	H. sang.	0.22	0.9	0.41	0.6	0.21	0.7	0.02	2.7	0.07	4.0	0.93	8.9
	A. forb.	0.95	3.4	2.17	6.1	1.48	3.4	0.06	0.2	0.65	0.3	5.31	13.4
	A. forb.	0.91	2.7	1.66	3.0	2.20	0.3	0.11	0.1	1.08	0.2	5.96	6.3

Glucose was handled quite differently by the cells than were the amino acids. By the end of the first hour essentially all the glucose taken up was still unbound (Fig. 2). After 8 hours nearly 60% of the total quantity absorbed remained extractable in *Asterias*. (The calculated values for *Henricia* are insignificant because of the great amount of individual variation resulting from the apparent feeding behavior exhibited by some of these specimens.) Whether or not more of the glucose became bound cannot be determined from the data, since in the 3- and 20-day specimens there was a progressive loss of radioactivity, practically all of which appeared to be from the unbound material. The progressive disappearance of the

unbound glucose seems to suggest that this fraction was the first to be metabolized and lost as CO_2. Again, some exchanges possibly could have occurred between the two fractions.

Discussion

These experiments complement earlier work on the utilization of exogenous nutrients by starfish and confirm that at least two very different species of these animals possess biochemical mechanisms which enable them to remove various types of amino acids and glucose from sea water. These mechanisms apparently are efficient in picking up nutrients from even very dilute solutions. The limits of effectiveness of the absorptive machinery, however, have not been determined. Neither has much evidence yet been gathered concerning its chemical and physical properties.

While absorption probably can occur over all areas of the body surface, the greatest activity takes place in the oral region. This locality doubtless has the largest area of free surface, and very likely is more exposed to circulation of water than the other parts. It includes the tube feet, that protrude into the medium, and the entire region of the ambulacral groove, which is probably efficiently ventilated by means of ciliary tracts. Such tracts have been described repeatedly in various species, including *A. forbesi* (Budington, 1942) and *H. sanguinolenta* (Anderson, 1960; Rasmussen, 1965).

The full significance of the epidermal absorptive process is still uncertain. If Pequignat (1966) is correct in his conclusion that epidermal digestion by skin glands is a common phenomenon in starfish, one would expect the organisms to possess adequate mechanisms for the absorption and utilization of the different kinds of products released by such action. While the present investigation has indicated that some types of amino acids and glucose may be taken up through the epidermis, it is still undetermined if all the myriad types of organic compounds which presumably would be released through such a digestive process could be handled. In fact, as mentioned previously, the pattern of uptake observed for the mixture of amino acids suggests that certain types, representing nearly a third of the mixture, may not be readily absorbed. Likewise, there is as yet no confirmation that carbohydrates other than glucose can be utilized. Further investigations are contemplated which will more fully evaluate the diversity of compounds which may be taken up by epidermal mechanisms.

During the 20-day period in which the animals were studied there was little, if any, indication that nutrients were passed on to the internal regions of the body from the absorptive sites on the body surface. Very small amounts of radioactivity were detected in the internal organs of a few of the test specimens after several days, but since little consistency was seen, this activity was probably due to unavoidable contamination of the separate samples. Also, a few specimens may have ingested some of the slime, mucus, and algae which accumulated on the walls of the holding tanks, and this material could have picked up a slight amount of radioactivity. In any case, as the values observed for the internal organs are too low to be credited with significance, it should probably be concluded that epidermal absorption functions almost solely for the benefit of the superficial tissues.

The apparent feeding reaction exhibited by some of the specimens of *H. sanguinolenta* in the glucose medium is most interesting. Anderson (1960) carefully studied the structure and function of the digestive organs of this species and concluded that its Tiedemann's pouches were a "hydrodynamic organ or flagellary pump of prodigious effectiveness" (p. 393). He showed that *Henricia* was primarily a filter-feeder and could take up and entrap such material as suspended *Mytilus* sperm. Feeding experiments were also performed on *Henricia* by Rasmussen (1965). These were more quantitative than Anderson's and served to confirm further the great efficiency of this animal as a particle-suspension feeder.

The present observations reveal that the flagellary feeding mechanism described by the above workers can also be effective in the utilization of dissolved nutrient materials of relatively low molecular weight. The pumping mechanism of *Henricia* is apparently so efficient that it "pays" the animal to take up solutions of nutrients, provided they occur in at least minimal concentrations. The most significant aspect of these observations, however, is not so much the uptake of the dissolved materials, but rather, the nature of the stimulus which caused them to be taken up. Although further verification is needed, the stimulus appears to have been the relatively higher concentration (when compared to that of the amino acids) of the glucose solution used. This was the only variable observed in the experiments other than the type of compounds themselves.

Doubtless, in nature these animals frequently encounter various kinds of dissolved nutrients in equivalent or even greater concentrations than those used in the experiments. Some of these probably come from the external digestion of relatively solid organic substrates. The stomach of *Henricia* is rather unique among starfishes in possessing numerous zymogen cells (*cf.* Anderson, 1960), which likely are a source of enzymes for such a process. In the present experiments specimens were often seen in an apparent feeding position, with their stomachs everted as button-like protuberances applied against the algae-covered aquarium wall or between the valves of a gaping clam. Under normal circumstances, digestive products released during this activity would probably set off the pumping process. As the glucose in the experiments seems to have elicited the same response as the natural stimulus, one can conjecture that encounter by the animal of a significant concentration of dissolved nutrients in its environment could serve also as an effective stimulus for initiating the pumping process. Once pumping is started, the soluble nutrients are efficiently taken up into the internal digestive organs.

Henricia, then, seemingly obtains its nutrition through several different processes. It depends primarily on the suspended and dissolved materials normally present in the environment, but probably also can digest some solid food outside of its body. These nutritional substances apparently are taken up by means of flagellary currents, and absorbed internally, or, at least in part, are directly assimilated by the superficial tissues of the body which are also exposed to the substances.

An uptake of labeled glucose into the digestive system of *Asterias,* comparable to that observed in *Henricia,* was not noted. This difference in behavior probably was due to the fact that *Asterias* is primarily a predator. While it lacks the complex pumping apparatus possessed by *Henricia,* it does possess ciliated surfaces on its stomach. It relies on currents produced on these surfaces to bring in concentrated solutions of nutrients from victims digested externally by enzymes supplied from the

digestive glands *via* gutters in the stomach wall (*cf.*, Anderson, 1954). This process is probably not altogether different from the pumping of *Henricia.* In a previous note (Ferguson, 1963b), for example, I reported the uptake into the digestive organs of *Asterias* of C^{14}-labeled glucose and amino acids which had been injected into small clams just before they were fed to the starfish. In that case, the presence of the solid pieces of food appears to have stimulated the animals to activate their feeding mechanism. As in *Henricia,* once feeding was initiated, uptake of the dissolved materials into the digestive organs proceeded rapidly.

The probability that epidermal absorption of exogenous nutrients is a continuous process while normal feeding is generally a discontinuous one is perhaps quite significant. In a sense, the two activities may balance each other as sources of nutrition over a period of time. If such is the case, the internal regions of the body may be seen as receiving nutrition almost exclusively *via* the digestive tract, while the more external tissues would be nourished to a considerable extent directly through the epidermis. One might suppose, then, that if an animal were prevented from utilizing either one of the sources, it probably could not survive. In this vein, investigations have shown that various species of starfishes can live long periods with little or no visible food, but they cannot subsist indefinitely under such conditions (Galtsoff and Loosanoff, 1939; Vevers, 1949). It would be much more difficult to design an experiment in which specimens were allowed to eat but completely denied epidermal absorption. But since it has been determined that epidermal absorption of nutritional materials does occur, and can take place to a significant degree, it seems reasonable to conclude that such absorption is an important factor in the economy of these organisms.

Summary

1. Small specimens of *A. forbesi* and *H. sanguinolenta* were exposed to dissolved C^{14}-amino acids and glucose. The subsequent distribution of these materials was then determined in the following five regions of the body: disk (including the gonads), oral body wall of the rays, aboral body wall of the rays, stomach, and digestive glands.

2. In all cases, large proportions of the labeled nutrients were taken up into the external tissues. The largest amount was usually absorbed into the oral body wall, which probably possesses a proportionately greater ventilated surface area than the other regions.

3. Over a period of 20 days there was little indication of movement of the externally absorbed nutrients into the internal organs. In this period, very little loss of amino acid radioactivity was noted. The amino acids became progressively less soluable in alcohol, suggesting that they were incorporated into the structural proteins of the organism.

4. Glucose radioactivity declined progressively over the 20-day period. As observed in *Asterias,* this decline occurred almost exclusively in the portion of absorbed glucose that remained alcohol-soluble. This fraction was possibly used as an energy source while the insoluble fraction became incorporated into more inert elements.

5. A number of the specimens of *Henricia* appeared to pump up and absorb the glucose medium into their digestive organs. This was interpreted as a form of

feeding behavior possibly initiated by the relatively high concentration of glucose used. The much less concentrated amino acid medium failed to initiate such a reaction.

6. It is concluded that nutrition in starfish is probably a dual process involving both a continuous epidermal absorption of dissolved exogenous materials for the benefit primarily of the superficial tissues, and intermittent oral feeding to satisfy the more general needs of the entire organism and especially of the internal organs.

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